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Effect of pre-existing *Schistosoma haematobium* infection on *Plasmodium berghei* multiplications in imprinting control region mice

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ABSTRACT

Objective: To investigate the effect of pre-existing *Schistosoma haematobium* (*S. haematobium*) infection on malaria disease severity.

Methods: The study involved the use of twenty-five imprinting control region mice, fifteen of which were initially infected with *S. haematobium*. Five of the remaining ten schisto-uninfected mice together with five schisto-infected mice were infected with *Plasmodium berghei* (*P. berghei*) after four weeks (acute stage) of schistosoma infection. The remaining five schisto-uninfected mice together with five schisto-infected mice were also infected with *P. berghei* after seven weeks (chronic stage) of schistosoma infection. The last five schisto-infected mice were used as control group. They were then monitored for changes in *P. berghei* parasitaemia on Days 3, 5, 7, 9 and 11 post-infection. Records on their survivability were also taken.

Results: The co-infected mice had significantly higher malaria parasitaemia, compared with the mono-infected mice during acute *S. haematobium* infection. In contrast, the co-infected mice had significantly lower malaria parasitaemia during chronic *S. haematobium* infection and a higher survival rate.

Conclusions: Co-infection of mice with *P. berghei* during acute *S. haematobium* infection resulted in rapid *P. berghei* development and increased malaria parasitaemia. However, the co-infection resulted in slower *P. berghei* development and decreased malaria parasitaemia with enhanced survivability of the mice during chronic *S. haematobium* infection. Therefore, pre-existing chronic *S. haematobium* infection may provide some protection to the host by reducing parasitaemia.

1. Introduction

Schistosomiasis and malaria are the world's two most important parasitic infections in terms of distribution, morbidity and

mortality[1]. Schistosomiasis and malaria are among the parasitic infections that share common transmission areas in various tropical regions. Recent studies in vertebrates have indicated that interactions between co-infecting parasites can be pronounced and have important consequences for disease development, severity and transmission dynamics[2,3]. Therefore, there is the need to determine how a given parasite will interact with another in the same host. It will show whether the presence of one parasite in the host hinders the activities of the other.

Although interactions between helminthes and malaria parasites

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could affect both parties, research has mostly focused on the extent to which helminth co-infection influences the malarial disease. In the past few years, studies have been conducted to elucidate the immune mechanism(s) involved in worm and malaria co-infections[4,5]. However, many of these studies have produced conflicting results, which has made it difficult to clearly understand the outcomes of these co-infections[5-14]. Some studies have reported an increased incidence of falciparum malaria in hosts with *Schistosoma mansoni* (*S. mansoni*)[6,10], while other studies have indicated that *Schistosoma haematobium* (*S. haematobium*) provides some protection from malaria such as lower parasitaemia and lower incidence[7-9].

Speculations on how helminthic infections may alter the susceptibility to clinical malaria have led to an increasing interest in investigating the consequences of co-infection. These studies have yielded contrasting results. Earlier studies reported a decreased malaria parasitaemia in co-infected mice whereas a recent one reported that mice with ova producing *S. mansoni* infection had increased malaria parasitaemia with *Plasmodium* infection[8,10-13].

Most studies that examined naturally occurring co-infection in humans indicated that co-infection with *schistosoma* and malaria parasites had an effect on the host, both in terms of pathological and immunological responses[14]. The direction of this response seems to depend on the host age, the malaria parasitaemia, the species of schistosome and the worm burden. Co-infection by these two parasites may have an important influence on the regulation of the immune response associated with the development of these infections and their respective morbidity[5,15,16].

Over the years, it has been more progressively speculated that helminth infections may change vulnerability to clinical malaria, and there is now escalating interest in investigating the repercussion of co-infection, with studies producing contrasting results[7-13]. Therefore, this study seeks to investigate the effect of pre-existing *S. haematobium* infection on malaria severity, using experimental animal model. Findings from our study will help to provide explanation to the observed trend of vulnerability or protection that pre-existing infection offers to new infections.

2. Materials and methods

2.1. Source of imprinting control region (ICR) mice

Male ICR mice, four weeks old, were purchased from the Noguchi Memorial Institute for medical research animal's house. They were kept in the animal facility at University of Cape Coast. Mice were maintained in ventilated cages and supplied with standard food and distilled water. The mice were randomly assigned to five groups, with each group containing five mice. The studies were conducted in accordance with accepted principles for laboratory animal use and care (EU directive of 1986: 86/609/EEC). Approval for this study was obtained from the Department of Biomedical and Forensic Sciences Ethical Committee.

2.2. Source of the helminthes and parasites inoculation

Infected *Bulinus truncatus* snails were collected from Baafikrom, a town near Mankessim, Central Region, Ghana. The snails were exposed to sunlight for about 30 min to shed their cercariae into clean water in Petri dish and checked for the presence and species of

the cercariae using a dissecting microscope at 40× magnification to discriminate *S. haematobium* cercariae from bird and other animal schistosomes. Cercariae from different shedding dish (Petri dish) were pooled into test tubes and centrifuged at a low speed of 800 r/min (revolution per minute) using Centrifuge 5702 R to concentrate the cercariae and allow mixing of sexes to avoid the possibility of single infection. The sediments were then collected and used to infect the mice. About 50-100 cercariae were percutaneously injected into each mouse with 2 mL syringe, for three groups of mice (Groups 1, 2 and 3). Groups 4 and 5 mice were not infected with cercariae. Helminth infection was confirmed by the presence of worms by portal perfusion after four weeks[17].

Plasmodium berghei (*P. berghei*) was provided from Noguchi Memorial Institute for medical research (Legon, Ghana). Parasites were stored as frozen stabulates at -80 °C. To obtain experimental inocula of *P. berghei*, packed red blood cells were passed through four donor mice. Infections were initiated in ICR mice by intraperitoneal injection of 0.2 mL of blood (packed red blood cells) containing 1.0×10^6 of *P. berghei* after four weeks of *S. haematobium* infection for Group 1 mice and Group 4 mice. After seven weeks of *S. haematobium* infection, Groups 2 and 5 mice were also inoculated with 1.0×10^6 of *P. berghei*. Group 3 mice served as control for the chronic infection. The various treatment groups were monitored for parasite development and growth in the mice[18].

2.3. Parasitaemia determination

Thin blood films from tail blood were made using standard microscope slides on Days 3, 5, 7, 9 and 11, air-dried and fixed in an absolute methanol for 5 min. The stained blood films were observed under a standard light microscope with 100× oil immersion lens. Infected and uninfected erythrocytes in different fields of view were identified and counted. Infected red blood cells were counted microscopically in at least five microscopic fields, each showing approximately 300 cells[10,17].

2.4. Mice survivability

Mice were monitored daily for mortality, to evaluate the survival rate of schistosome mono-infected, *P. berghei* mono-infected and the co-infected mice for both acute and chronic schistosome infection.

2.5. Statistical analysis

Differences in the parameters measured for the different test groups were tested for significance by the independent *t*-test using SPSS 17 computer software. Differences in between groups were considered significant when *P* value was less than 0.05. Percentage survivability were also used as comparing parameters for the different groups.

3. Results

3.1. Survivability

During the chronic co-infection, the *P. berghei* mono-infected group of mice survived until Day 5 after which they began to die on Day 6. Only 40% of them survived until Day 8 after which they also died before Day 11. On the other hand, the *Schistosoma-Plasmodium* co-infected group survived until Day 6 when they started to die.

Eighty percent of them survived until Day 12 after which they all died by Day 14 (Figure 1). In contrast, all mice in schistosome mono-infected group survived during the entire experiment (Figures 1 and 2).

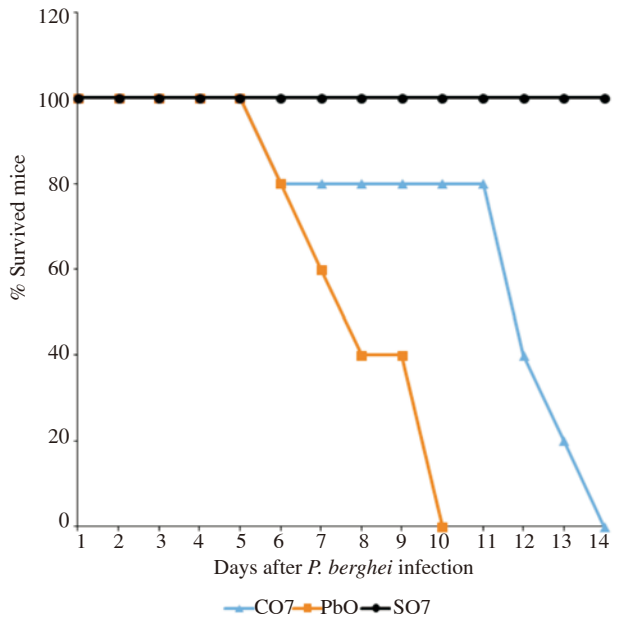


Figure 1. Length of survivability in *Schistosoma-Plasmodium* co-infected, *P. berghei* mono-infected mice and *S. haematobium* mono-infected mice during chronic schistosome infection. CO7: 7th week co-infected group; PbO : *P. berghei* mono-infected group; SO7: 7th week *S. haematobium* mono-infected group.

However, during the acute co-infection, the co-infected mice started to die on Day 7, whilst the *P. berghei* mono-infected mice started to die on Day 5, with all mono infected mice dying by Day 10 after *P. berghei* infection. But, all the co-infected group died by Day 11 (Figure 2).

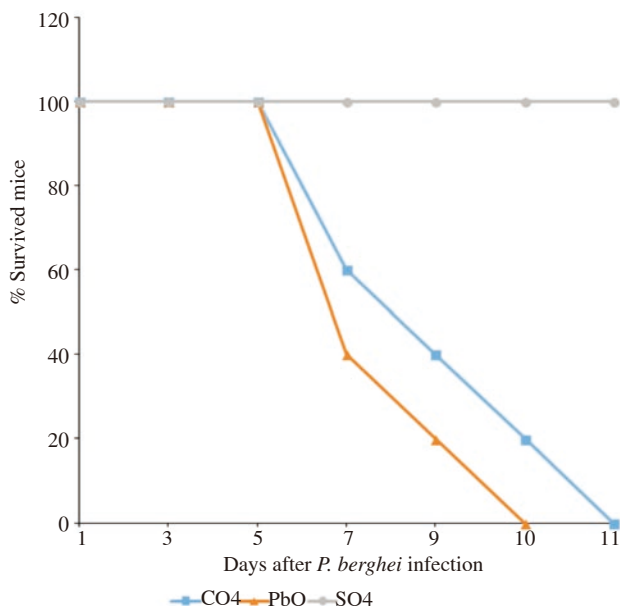


Figure 2. Length of survivability in *Schistosoma-Plasmodium* co-infected, *P. berghei* mono-infected mice and *S. haematobium* mono-infected mice during acute schistosome infection. CO4: 4th week co-infected group; PbO: *P. berghei* mono-infected group; SO4: 4th week *S. haematobium* mono-infected group.

3.2. Malaria parasitaemia

During the acute stage of infection, malaria parasitaemia was determined on Day 3 in both single and co-infected group. On Day 3 after *P. berghei* infection, the *Plasmodium* mono-infected group developed a parasitaemia of 10.70% ± 0.26% whilst, the co-infected group developed a parasitaemia of 18.60% ± 0.21% ($P < 0.01$). The parasitaemia increased to 28.75% ± 0.19% and 23.78% ± 0.17% at Day 5 for both the co-infected and *Plasmodium* mono-infected group respectively ($P < 0.05$). However, the parasitaemia peaked at Day 9 for both groups with 52.61% ± 0.19% and 51.71% ± 0.15% for the co-infected and mono-infected mice ($P < 0.05$). All the infected mice were dead by Day 11, thus no parasitaemia was recorded for Day 11 during acute schistosome infection (Figure 3).

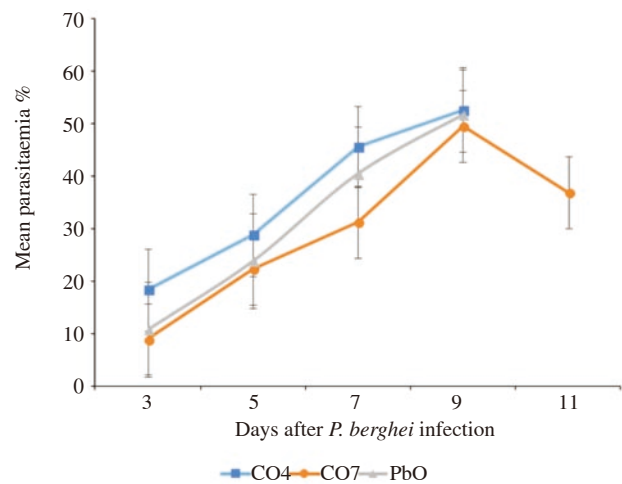


Figure 3. *P. berghei* parasitaemia in mice co-infected with *P. berghei* 4 weeks and 7 weeks post-*S. haematobium* infection and in mice infected with *P. berghei* only. CO4: 4th week co-infected; CO7: 7th week co-infected; PbO: *P. berghei* mono-infected.

Infection of mice with *P. berghei* seven weeks post-*S. haematobium* infection resulted in slower development of malaria parasite and decreased malaria parasitaemia than *P. berghei* mono-infected mice which was in contrast to the acute infections. The co-infected group of mice had parasitaemia of 8.95% ± 0.63% on Day 3, compared to 10.70% ± 0.74% in the *P. berghei* mono-infected ($P < 0.05$). At Day 7, the co-infected group developed a parasitaemia of 31.24% ± 0.05% compared to that of the mono-infected group of 40.5% ± 0.27% ($P < 0.01$). Both, co-infected and mono-infected mice recorded a peak parasitaemia of 49.58% ± 0.24% and 51.72% ± 0.28% respectively ($P = 0.032$) at Day 9. Finally, the co-infected mice experienced a reduction in parasitaemia (36.91% ± 0.40%) on Day 11. However, all the mono infected mice were dead by Day 11 (Figure 3).

4. Discussion

Many studies have been carried out to examine the impact of *S. mansoni* infection on malaria severity, both in mouse models and in humans, yet the results are limited and often conflicting. In this study, our aim was to investigate the effect of pre-existing *S. haematobium* infection on malaria disease severity in mice. This is also the first study in Ghana to document the impact of acute and chronic pre-existing *S. haematobium* infection on malaria disease severity in mice.

In this study, mice with 4 weeks (acute stage) *S. haematobium* post infection with *P. berghei* co-infection favoured rapid *P. berghei* development and increased *P. berghei* parasitaemia than mice with *P. berghei* infection only. However, mice with 7 weeks (chronic stage) *S. haematobium* post infection with *P. berghei* co-infection was found to slow down *P. berghei* development and reduced *P. berghei* parasitaemia than mice with *P. berghei* only. This is in agreement with other studies which indicated that *S. haematobium* provided some protection from malaria[5,7,8]. This finding is also in agreement with observation by Christensen *et al.*[19], who reported that the outcomes of experimental co-infections with protozoan and trematode parasites depended on the parasite species as well as the relative timing of infection. However, the findings is in contrast with a number of previous studies which have demonstrated higher malaria parasitaemia in mice co-infected with various species/strains of rodent malaria and worms than those infected with the malaria parasite alone. For instance, Bucher *et al.*[10], reported increased malaria parasitaemia during early stages of malaria in chronic *S. mansoni*-*P. berghei* co-infected mice than mono-infected mice.

The rapid *P. berghei* development observed during the acute stage of *S. haematobium* infection in the co-infected group of mice appears to indicate that the acute post *S. haematobium* infection predisposes the host to more stern infection. The elevated *P. berghei* parasitaemia seen in *S. haematobium*-*P. berghei* co-infected mice could be attributed to a schistosome parasite induced Th2-mediated immune inhibition of the *P. berghei*-induced Th1 response. This corroborate with findings that malaria resistant mice have Th1 type protective immune response against blood-stage *P. berghei* infection[20]. Lyke *et al.*[9], revealed that the polarized Th2-enriched environment induced by underlying schistosomiasis modulated the human immune response, possibly affecting the incidence and severity of concomitant falciparum malaria. The observed increase in parasitaemia in the co-infected group of mice may delay parasite clearance during treatment[21].

Our understanding of the mechanisms by which interactions between parasites occur is still limited. Recent studies have proposed an immunologic hypothesis based upon the T cell dichotomy[22-24]. If one considers that for each parasite or

pathogen, there is a corresponding protective T cell/cytokine response; concomitant infection could lead to synergistic or antagonistic T cell responses, dependent upon what kind of response (Th1 or Th2) was induced by each parasite[20,22-25]. Synergistic responses could decrease the pathologic impact of the infections, whereas antagonistic responses could exacerbate the diseases[8]. The nature of immune responses may vary according to the stage and intensity of infections[16,22-24]. This could explain the slower *P. berghei* development and decreased malaria parasitaemia observed during the chronic stage of *S. haematobium* infection. Therefore, the observed disparity in the parasitaemia may be attributed to the effect of nature of immune response mounted against the parasite by the host, which may vary according to the stage and intensity of infections[19,23-25].

One interesting finding of this work is that post-infection survivability of mice co-infected with *S. haematobium* and *P. berghei* enhanced survival of mice. The co-infected mice survived longer than the mono infected mice. The observed disparity in our experimental outcomes from different studies may have resulted from differences in *Plasmodium* and schistosome species used in the various studies, the potency of *S. haematobium* during co-infection and differences in the dose of *S. haematobium* cercariae used to infect the mice. Although Bucher *et al.*[10], reported that the presence of chronic *S. mansoni* infection did not influence the survival of *P. berghei* infected mice. The data obtained in the present study showed that chronic *S. haematobium*-*P. berghei* co-infected mice survived for longer period of days than the *P. berghei* mono-infected mice.

The co-infection of mice with *P. berghei* during acute *S. haematobium* infection resulted in rapid *P. berghei* development and increased in parasitaemia accompanied with enhanced survivability of the mice. However, co-infection of mice with *P. berghei* during chronic *S. haematobium* infection resulted in slower *P. berghei* development and decreased malaria parasitaemia but similarly enhanced survivability of the mice. Therefore, pre-existing chronic *S. haematobium* infection may provide some protection to the host by reducing parasitaemia. We recommend that further studies are done to measure some immune parameters to clarify the immunoregulatory mechanism that occurs during the process of co-infection.

Conflict of interest statement

We declare that we have no conflict of interest.

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